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1: The physiology of nutrition and growth

2: Experiments to influence the cytology of Protozoa

3: Pathology and Protozoa

4: Latent infections

5: Artificial Parasitism in Protozoa

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Protozoa as Model in Biological Research.

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Simultaneously with our growing knowledge of the morphology, physiology and ecology of Protozoa, these organisms are being used as model for investigating various elementary biological processes. For many reasons, Protozoa are particularly suitable for experiments of this kind. In spite of their microscopic dimensions, most of them, when magnified 1000 times, permit us to study their morphology. Composed of one single cell, they already constitute independent organisms corresponding in their general behaviour with that of primitive multicellular organisms, enabling us to study exogenous influences in individual forms as well as in entire populations. Their methods of sexual reproduction vary. Of special significance are those Protozoa who cause serious, often fatal diseases in man as well as in domestic animals.

Twenty years ago, in 1941, CALKINS-SUMMER in their work PROTOZOA IN BIOLOGICAL RESEARCH summarized all that was then known about the morphology, physiology, biochemistry and biophysics of Protozoa. Since then, experimental research methods have been greatly perfected /we are using isotopes, to quote just one example/, and the importance of Protozoa has increased in every respect. Allow me to refer to a few instances in the solution of which we have also participated.

1/ The physiology of nutrition and growth

Serious investigations of the physiology of the nutritional habits of Protozoa could be started only after the scientists had succeeded in producing pure cultures - i.e. cultures free of associating bacteria - of at least the main representatives of the individual protozoan groups and other microorganisms, preferably in media of a known chemical composition. It was comparatively easy to produce pure cultures of Protozoa living in naturally sterile environments. Thus, for instance, the first pure Trypanosoma cultures were produced as early as 1903 from the blood of birds and, later on, also from the blood of mammals /NOVY-McNEAL/. To produce pure cultures of Protozoa living in bacteria-filled environments, such as in the intestines or in brackish water, was a much more complicated task. Sometimes the Protozoa themselves came unintentionally to the help by passing from the intestines into the blood where they were easier to isolate /Eutrichomonastix colubrorum 1918/. On another occasion the amount of bacteria was so strongly reduced by mechanical means that they multiplied much slower than the Protozoa and were devoured by them /for instance, in the case of Mayorella palestinensis/. To free Protozoa from bacteria various methods were devised including washing, migration, phototaxis, etc. Much was done in this respect by PRINGSHEIM and LWOFF et all. The discovery of antibiotics, particularly of penicillin and streptomycin, marked a fundamental turning point in pure culture production. These antibiotics prevent the multiplication of a number of bacteria and make it possible to reproduce, after a few transoculations, pure cultures also of those Protozoa which live in strongly contaminated environments. Of many protozoans, especially of the ciliates which require formed food, it was possible to produce "Zweigliederige Kulturen", i.e. carnivores feeding on living or dead bacteria, yeast or other smaller protozoan species. Finally, pure culture production of some Protozoa so far succeeded only in tissue cultures of cells from higher vertebrates, e.g. Toxoplasma, Plasmodia etc.

It was only by working with pure cultures that the basic proteins, aminoacids, carbohydrates and growth substances as well as "trace" elements which are absolutely necessary for the growth and reproduction of protozoans could be determined. Some species could also be used to ascertain some vitamins, for instance aneurin /by means of Strigomonas and Tetrahymena/, vitamin B-12 /by means of Euglena and Ochromonas/ etc.

Protozoa served as valuable indicators in determining the quality of non-drinking water /for inst. in the saprobic system after KOLKWITZ-MARSSON/. The role of Protozoa in water is not confined to devouring bacteria and smaller protozoan species but it provokes also certain mutual stimulations and antagonism between the different species with all the implications for self-purifying processes in the water. Unfortunately, our knowledge regarding the physiology and particularly the metabolism of even the very well-known protozoan species is still very limited.

2/ Experiments to influence the cytology of Protozoa.

The complicated protozoan body calls for experiments aimed at artificially influencing the cell function by removing various organelles or changing them through external stimulation. Experiments to regenerate Protozoa are generally known and have shown that the presence of the nucleus is inevitable for the existence of the body. The first cell organelle ever to be removed was the blepharoplast of Trypanosoma. It had been known before that certain species of Trypanosoma are not equiped with blepharoplasts /Trypanosoma equinum/ and that in numerous other species possessing blepharoplast 1 - 10 per cent of the organisms have no organelle of this type. By means of trypaflavine and other dyes blepharoplasts of Trypanosoma could be removed directly in the hosts /WERBITZKI 1910 and others/ and strains permanently free of blepharoplast were produced. There were no detrimental effects on their virulence, reproduction power, motility etc. It is still dubious how the blepharoplasts disappear - whether this is due to a loss of reproduction power, to decomposition or a natural selection of organism void of blepharoplasts. Elimination in vitro from the body of Trypanosoma has so far not met with success.

Another example of artificially influencing the cytology and at the same time the physiology of protozoans is the elimination of the photosynthetic apparatus and hence the faculty of the flagellate Euglena gracilis to assimilate. ZUMSTEIN's experiments in 1900 were actually the first attempts to influence the cytology and physiology of the flagellates - Zumstein at that time discovered that Euglena gracilis placed into permanent darkness lose their colour but continue to grow in this apochlorotic state which greatly influences the condition of their chloroplasts. The fact that the elimination of the photosynthetic apparatus of Euglena gracilis may be achieved by using streptomycin /PROVASOLI et all. 1948, JÍROVEC 1949/, by means of antihistamine, e.g. pyribenzamin /GROSS et all. 1955/, etc. as well as by physical methods, for inst. through higher temperatures during the reproduction process /PRINGSHEIM 1948/, UV-rays /PRINGSHEIM 1958/ etc., was a finding of utmost significance. Euglena "bleached" in this fashion proved to be analogous to the free-living flagellate Astasia longa. The nature of this phenomenon has so far remained unexplained; some attribute it to a retarding of plastid reproduction during cellular fission, others ascribe it to the loss of the capability to build up chlorophyl and the transformation of chloroplasts into leucoplasts, others again regard it as fragmentation and a general disappearance of chloroplasts. Recently De DEKKEN-GRENSON stressing the interesting analogy between the bleached Euglena and the origin of respiratory defficient Saccharomyces cerevisiae colonies maintained that the disappearance of the photosynthetic apparatus is due to the accumulation of some metabolit or other or to a change in the metabolism giving rise to permanent hereditary transformation. Another organelle to be externally influenced is the stigma in Euglena gracilis which disappears in some Euglena strains when streptomycin or other factors are applied, or it is preserved after the lost of chloroplast, providing the cultures are placed in the light. The photoreceptor connected with the eyespot disappears simultaneously with the stigma. VÁVRA, however, has shown that in one strain the photoreceptor was preserved even after the loss of stigma.

Experience hitherto has shown that individual strains or colonies of one or the other species react very differently in experiments to influence their cytology and that only a large amount of experimental material can yield satisfactory results.

Also transplantation of the nucleus into organisms of identical or different species made possible by the improvement of micrurgical techniques are highly interesting. As early as in 1939 COMANDON and DE FONBRUNE exchanged the nuclei of two specimens of Amoeba sphaeronucleus. After removal of their own nucleus the amoebae continued to live normally with the new nucleus. DANIELLI and LORCH /1950/ exchanged the nuclei of Amoeba proteus and Amoeba discoidoliswho differ somewhat in their morphology, manner of movement, rhythm of fission etc. It became evident that the cytoplasm decisively influences the shape of amoeba, its movement and even the size of the transplanted nucleus. The nucleus again determines the rhythm of division and the antigene properties. We feel justified in expecting many surprises from the future transplantation experiments.

The event of electron microscope opened new progress in the study of internal structures of Protozoa. Besides enabling us to investigate the structure of the hitherto known organelles /mitochondria, Golgi apparatus, flagella, filaments/ it also led to the discovery of new organelles /toxonemes, sarconemes etc./. It is to be expected that with the help of electron microscope the studies of the various artificially induced changes will considerably enlarge our knowledge of experimental cytology.

3/ Pathology and Protozoa.

I do not have to dwell on the generally known importance of Protozoa in the human and veterinary medicine, nor on our achievements in the present struggle to eliminate various infections caused by Protozoa which until recently were among the most wide-spread contagious diseases in the world. The complex battle waged against the parasites by means of modern chemotherapy, and against their carriers by means of insecticides, a detailed knowledge of the development cycle of the parasites and the application of Academician Pavlovsky's teaching pertaining to the natural centres of infection /e.g. leishmaniosis, borreliosis etc./ have all led to the retreat of protozoans in large regions, particularly in tropical zones. The best example of this is the eradication of malaria, formerly one of the most wide-spread contagious diseases in the world, in all those tropical and subtropical regions which have at least to some degree access to civilisation. Basic investigation of the parasitic protozoans also played an important role in this respect. I should like to cite a few examples.

The tissue fibres of some parasitic Protozoa which cannot exist without a host cell are valuable material for the investigation of toxoplasmosis, American trypanosomiasis and other diseases. Model cultures facilitated the study of metabolism of parasites as well as the influence of various chemotherapeutics. In this connection I should like to mention the surprising discovery of Acanthamoeba in tissue cultures of monkey kidney /CULBERTSON 1959/. Hitherto known as a free-living form this amoeba proved to be strongly pathogenic in mice and monkeys creating large deposits in their brains and lungs.

A new branch of science, chemotherapy, owes its existence to experiments in which parasitic Protozoa were used as models. Not meeting with success for a long time in combating bacterial infection chemotherapy was subplanted by serotherapy, but absolutely won the field in fighting against parasitical infections. Trypanosomes and Plasmodia easily cultivated in small laboratory animals permitted serial experiments in which the chemotherapeutical effect of large quantities of compounds could be studied - the result of this effort was the discovery of Atoxyl, Salvarsan, Trypanosamid, Germanin, Pentamidin, Atebrin, Plasmochin, Daraprim, Paludrin, Chlorochin and other preparations often of a surprising effectiveness. WAGNER-JAUREG's discovery of malaria therapy made it possible to work out and study the course of model infections in psychiatric hospitals /for inst.CIUCA and others in Rumania/, which considerably contributed to the successful fight against malaria in the field of curative medicine. Discovery of E-stages, first in bird later also in human plasmodia /HUFF, RAFFAELLE, KIKUTH, REICHENOW-MUDROW, GARNHAM and others/ helped to understand hitherto unknown laws regarding the epidemiology and pathogenesis of malaria. Finally, the discovery of Plasmodium berghei and Plasmodium vinckei in small African rodents and their successful transfer to ordinary laboratory animals facilitated further immuno-biological and chemotherapeutical research.

If we were so far satisfied with general ascertaining whether or not certain substances have an effect on parasites, today we are already engaged in a more detailed research of the effect of those substances on various metabolic processes in the body of parasitic Protozoans and their hosts. It is conceivable that only the results of experiments with new drugs applied directly to the contaminated host can be considered valid for an application in practice and for the determination of their ... effects. It was shown by JÍROVEC /1947/ that cultures of the infusorian Tetrahymena pyriformis and other Protozoa are suitable for experiments to establish the toxicity of various chemotherapeuticals and desinfectants for animal cells in general. Thus penicillin and streptomycin proved to be non-toxic for these protozoans as well as for animal cells in general, whereas Patulin, Merfen etc. were highly toxic. Aureomycin, Terramycin, Chloramphenicol and others can be described as of a medium toxicity. It is therefore possible to substitute for inst. Tetrahymena cultures when testing the toxicity of some substances.for.

4/ Latent infections.

The research during the last 30 years has proved that Protozoa can seriously endanger the health of man and domestic animals also in the mild climate. Toxoplasmosis and vaginal trichomoniasis are among the most wide-spread protozoan infections of man while domestic animals are very frequently infected with coccidiosis.

In recent years an important change took place regarding the opinion on the pathogenesis of certain infectious diseases. While under R.KOCH´s influence we believed that the meeting of an infective agent and a suitable host is bound to be followed by the outbreak of a disease, we know today that the host-parasite relations are much more complicated than that and that in a considerable number of infections the manifest clinical sickness only represents the terminal phase in this relation.

From the practical point of view this phase is certainly a most important one, but it is merely one of two extremes, the second of which is the latent infection. Prevalence of one extreme or the other depends on several factors : virulence of the parasite which can considerably vary even within individual strains of the same species, resistence of the host which changes with its age, his nutritional habits, hormon cyclus and sometimes even the quantity of germs present. This applies e.g. in the case of coccidiosis and also probably of toxoplasmosis. I should like to mention some model infections in order to explain the importance of these latent processes which hitherto received only minor attention.

Toxoplasmosis is an anthropozoonosis and one of the most widespread protozoan infections in man. On the basis of the positive serum reactions and intradermal tests it can be estimated that on the average 25 per cent of the Central European population are infected and that the highest number of infected persons is found in the higher age groups. Compared with this figure the amount of clinically diagnosed toxoplasmosis, pre-natally or post-natally acquired, is negligible, even though in the recent years major attention was paid to this disease in the clinics and research laboratories. The first case of ophthalmic and congenital toxoplasmosis in man was described by Professor Janků in 1923 in Prague. This means that there exist on the one hand serious and often fatal Toxoplasma gondii infections in man and animals, on the other hand, infections which are entirely latent. For these reasons we feel justified in presuming the existence of a whole number of transitional stages between the two extremes, with very different clinical symptoms as to their quality and significance. A decisive factor is probably also the quantity of parasites present, the resistance of the host and the localisation of parasites persisting in the body. Meanwhile it is becoming ever more clear that the latent toxoplasmosis plays an important part in causing various disorders in the development of the human embryo, habitual miscarriages and other disturbances in expectant mothers. In ophthalmology granular chorioretinitis is often diagnosed, in internal medicine we know a number of diseases of the lymphatic system and the blood vessels which, of course, require further investigation regarding the connection between the actual or potential latent infection and certain clinical symptoms. In these experiments artificially infected laboratory rats also serve as model for human infection. The toxoplasmic infection, for instance, remains latent in laboratory rats as well as in human beings, while contaminated mice, guinea pigs etc. mostly die of the infection. The study of disturbances in animal development as a result of toxoplasmic infections offers a wide field of activities for comparative teratology.

Pneumocystosis is another wide-spread and in most cases latent infection in man and certain animals. At the beginning we knew, of course, only one type of fatal pneumocystosis which affected babies of 2 - 4 months. Later, however, fatal Pneumocystis carinii infections were also detected in older children and in form of lung complications also in adults weakened by a long illness or a lung disease. Certain post-mortem examinations as well as positive serum reactions and intradermal pneumocystis antigen tests have shown that latent Pneumocystis occurs also in adults. The rate of infection increases again with the age and it is estimated that 2 - 5 per cent of middle-aged adults are affected by the latent infection, while in older persons living in old-age homes the percentage through the effects of living in a closed community jumps to almost 50 per cent. Accute illness, however, occurs only when the organism is weakened, either as the consequence of another illness or chronical disease /TBC, leukemia, lymphogranulom, lymphoblastom etc./. Isolated older finds of Pneumocystis in animals indicate wide-spread latent infections in domestic animals and wild mammals. This was proved by long-term applications of large doses of Cortison which provoked after several months a typical Pneumocystis pneumonia in 60 per cent of rats and 80 per cent of rabbits used in the experiments /WELLER, LINHARTOVÁ and others/. Toxoplasmosis and pneumocystosis can thus serve as model for changing the latency into manifest sickness.

Trichomoniasis is another protozoasis, affecting men and women in all parts of the world. The clinical symptoms of this disease were first diagnosed in women, while there were very few cases reported in men. It was clear to the protozoologists that Trichomonas vaginalis, a protozoan which is very sensitive to its environment and does not form cysts, which is unable to live in water or even dried-up medium, can, in the majority of cases, only be transferred by sexual intercourse. Only during the past 20 years the research succeeded in proving that infected women have, at least in the initial stages, typical clinical symptoms, while the infection in male patients, acquired of course, only by sexual intercourse, remains latent in most instances, or has such negligible clinical symptoms that they escape attention. On the whole, the percentage of latent infection in men corresponds with the percentage of infected women in a certain population.

Successful transfer of Trichomonas onto permenetly oestrogenized rats facilitates a number of experiments, regarding the pathogenesis and chemotherapy of this, at present most wide-sperd, venerel disease in men.

If, on the one hand, many parasitical protozoans cause grave concern, some, on the other hand, proved to be very useful to us. At present, though, the possibilities in this respect are still rather vaguely outlined and we have yet a long way to go before we shall be able to use them in practice on a large-scale. I am referring to the significant discovery by ROSKIN et all, which was corroborated by other scientists, regarding the cancerolytic effects of an extract won from Trypanosoma cruzi, as well as to other possibilities to fight against various parasitical insects by means of parasitical protozoa, a method which was suggested and already practically applied by WEISER /for inst. the use of the microsporidian Thelohania hyphantriae to combat Hyphantria.

5/ Artificial Parasitism in Protozoa.

Free-living Protozoa, for example Infusoria, were often detected in the body cavities of various water-dwelling animals, especially in insect larvae. LWOFF /1924/ was first to show that Tetrahymena, inoculated into the body cavity of the caterpiller Galleria melonella, multiplies rapidly and finally devours its host. In 1937, JANDA and JÍROVEC succeeded in infecting a large number of insects with this protozoan. All infected insects died within less than a month. Water-dwelling insects were infected by means of incissi cutiscula. Short-term heating at 32-35° C healed the infection permanently, but no immunity against reinfection resulted. Also the use of dead Tetrahymena did not lead to immunisation. ERHARDOVÁ, in 1952 ascertained that not only Tetrahymena but also the infusorians Colpoda steini, Colpodium colpoda, Glaucoma vorax, etc. are highly virulent for various kinds of Coleoptera, Lepidoptera and Rhynchotae. POKORNÁ, in 1961, obtained a fatal infection in the caterpillars Bombyx mori and Galleria Melonella, by injecting flagellates of the genus Strigomonas. Euglenas however, did not multiply in insect hosts at all. Hence, artifical parasitism of some infusorians is a suitable model experiment for to study the pathogenetic processes of an entirely unadapted host.

I could, of course, continue reporting many more, very interesting results of protozoan research, I could speak of the influence of various kinds of radiation /RTG, UV, Ra, etc./ of the discovery of kappa-particles in the plasma of Paramecium of the Killers type /SONNEBORN et all./ and of many other aspects. I am convinced that we shall hear many interesting papers, dealing with the genetics of Protozoa at this Conference and I have no doubts that modern biology in future will use protozoa as model organisms for the study of various biological processes on an even larger scale than hitherto.